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Abstracts

Evolution and Development

245

How many steps to build animals?

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All theories of the origin of the Metazoa begin with unicellular eukaryotes and end with the known phyla. Although the endpoints are thus fixed, the respective trajectories (pathways) of various theories through genotypic and phenotypic space do not agree. Using a formalization developed by Stadler et al. (2001), I present an analysis of the problem of the origin of metazoan form that characterizes the evolutionary problem to be solved in terms of finding pathways on which natural selection can act.

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246

Development of mandibular and clavicular secondary cartilage is strongly influenced by mechanical cues from the skeletal musculature

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We employed Myf5−/−:MyoD−/− fetuses that completely lacked striated myoblasts and myofibers to study bone development in the absence of mechanical stimuli from the musculature. We concentrated on development of the mandibles and clavicles. More specifically, we asked (a) how the secondary cartilage formation and other osteogenic events are initiated and maintained in the absence of the mechanical stimuli from the skeletal muscle and (b) to what extent and how the size and shape of the mandibles and clavicles is altered in the absence of the stimuli from the skeletal muscle. We employed morphometry and morphology at different embryonic stages and compared bone development in Myf5−/−:MyoD−/− and control fetuses. Our findings can be summarized as follows: (a) the mutant mandibles and clavicles had dramatically altered shape and size, (b) these effects varied depending on the bone (e.g., clavicles being more dependent than mandibles) and even within the same bone (e.g., different processes of the mandible), and (c) we further supported the notion that mammalian clavicles arise under different influences from that that initiate the wishbone in birds. Together, our data show that the development of secondary cartilage, and in turn the development of the final shape and size of the bones, is strongly influenced by mechanical cues from the skeletal musculature.

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247

Lamprey type II collagen and Sox9 reveal an ancient origin of the vertebrate collagenous skeleton

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Type II collagen is the major cartilage matrix protein in the jawed vertebrate skeleton. Lampreys and hagfishes, by contrast, are thought to have non-collagenous cartilage. This difference in skeletal structure has led to the hypothesis that the vertebrate common ancestor had a non-collagenous skeleton, with type II collagen becoming the predominant cartilage matrix protein after the divergence of jawless fish from the jawed vertebrates, approximately 500 million years ago. Here, we report that lampreys have two type II collagen (Col2α1) genes that are expressed during development of the cartilaginous skeleton. We also demonstrate that the adult lamprey skeleton is rich in Col2α1 protein. Furthermore, we have isolated a lamprey orthologue of Sox9, a direct transcriptional regulator of Col2α1 in jawed vertebrates, and show that it is co-expressed with both Col2α1 genes during skeletal development. These results reveal that the genetic pathway for chondrogenesis in lampreys and gnathostomes is conserved through the activation of cartilage matrix molecules and suggest that a collagenous skeleton evolved surprisingly early in vertebrate evolution.

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248

Genetic stabilization of vertebrate bilateral limb symmetry as an example of cryptic polarity

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A striking feature of vertebrate embryos is the bilateral symmetry of their exterior body plan, that is also reflected in the development of limb buds in pairs. Mendelian inheritance analyses now provide an unexpected link between two important topics of developmental biology, namely limb development and left–right asymmetry. Among teleost killifishes (Cyprinodontidae) some species completely lack a pair of pelvic fins (e.g., *Aphanius apodus* Gervais 1853), and they are homozygote for this character. To study the genetics of fin induction this species was crossed with closely related killifishes that possess pelvic fins (*A. iberus* Cuv. and Val. 1846). Astonishingly, a constant proportion of the hybrid offspring developed only a single pelvic fin. Also, in natural populations of *A. iberus*, specimens can be found having single pelvic fins on either the right or left side. Selective breeding of these single-finned individuals has revealed that fin laterality is not random but genetically controlled. The evidence for an independent genetic control of left- versus right-limb induction suggests the remarkable hypothesis that the development of vertebrate limbs in pairs is not a simple default state, as might have been be supposed by the idea of establishing bilateral mirror image appendages in pairs based on a single signaling source. Instead, a bilateral balanced genetic stabilization seems to be necessary to initiate every individual left and right limb bud. Therefore there must exist an unexpected mechanism for assigning left and right identity to seemingly identical structures (such as limbs), thus revealing a cryptic underlying polarity.

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249
Limb heterochrony in a marsupial, *M. domestica*
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Heterochrony, or a shift in developmental timing, is an important source for evolutionary change. Here, we utilize *Monodelphis domestica* (Metatheria), an opossum, as a model to investigate the developmental origins of limb heterochrony. *M. domestica* neonates show significant acceleration of forelimb (FL) development relative to the hindlimbs (HL) when compared to other non-metatherian amniotes. When the HLs of *M. domestica* embryos begin outgrowth, the FLs are well-defined buds, indicating that heterochrony arises very early, before outgrowth of either limb. There are at least three potential mechanisms by which this heterochrony might be produced. FL outgrowth could be initiated earlier than HL outgrowth by virtue of earlier genetic signaling to the FL field or later signaling to the HL field. Alternatively, a slowing of axis extension and cell proliferation in the primitive streak could limit tissue availability in the posterior lateral plate mesoderm (LPM), so that the field of cells that will eventually give rise to the HL field are not yet present when the FL begins outgrowth. Similarly, at the time of FL outgrowth initiation, the axis may not have extended far enough for the HL field to have been specified. That is, there may be LPM present that will eventually be specified as HL, but it does not yet have an anterior–posterior positional identity. In this study we provide in situ hybridization data to distinguish these mechanisms. We detail the expression patterns of *tbx4*, *tbx5*, and *fgf10*. Results suggest that slowed axis extension may limit tissue availability from the streak and delay the initiation of HL outgrowth.

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250
Comparative development of mammalian and alligator metapodial growth plate formation
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Mammalian metapodials, unlike most long bones, form only a single growth plate. We have shown that the mouse metatarsal growth plate displays a peak of chondrocyte proliferation corresponding to the ‘resting zone’ that contrasts with the more diffuse pattern at the non-physis end undergoing ‘direct ossification’. To determine if this particular mode of ossification and growth is unique to mammalian metapodials, we traced the development of the growth plate in the alligator (*Alligator mississippiensis*). We find that, unlike mammals, growth plates form at both ends of the alligator metapodial and are maintained in the subadult. Prior to growth plate formation, embryonic alligators differ from mice in showing poor columnar organization and gradual transition from columnar to hypertrophic chondrocytes, though once growth plates are established these parameters are largely similar. Immunohistochemistry reveals that key factors that regulate growth plate formation in mammals (Ihh and PTHrP) are also expressed in alligators. PCNA results show that during growth plate formation alligators exhibit the same proliferative peak in their presumptive resting zones as mice, indicating that this is potentially a key event in the development of a growth plate. The broad commonalities in mouse and alligator growth plates indicate that direct ossification of one epiphysis and reliance on a single growth plate are a novel adaptation in mammalian metapodials. Our analysis also shows that the anatomical locations of growth plates have been modified during vertebrate evolution. Supported by NSF and NIH.

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251
Effect of a single dose of ethanol on developing peripheral nerve of chick embryos
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Fetal alcohol syndrome (FAS), a condition occurring in some children of mothers who have consumed alcohol during